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Docket No.: NY-ROCHE 202-US

Application No. 10/634,477

Amendment dated April 24, 2008.

Reply to Ex parte Quayle Action of April 14, 2008

APR 24 2008

AMENDMENTS TO THE CLAIMS

1. (Previously presented) A method of treating disturbances in iron distribution in a patient suffering from non insulin dependent diabetes mellitus comprising administering to a patient suffering from non-insulin dependent diabetes and disturbances in iron distribution a therapeutically effective amount of human erythropoietin protein having the amino acid sequence of SEQ ID NO:1 sufficient to treat said disturbances in iron distribution.
2. (Canceled)
3. (Canceled)
4. (Canceled)
5. (Previously presented) A method of treating disturbances in iron distribution in a patient suffering from non insulin dependent diabetes mellitus comprising administering a therapeutically effective amount of human erythropoietin protein having the amino acid sequence of SEQ ID NO:1 modified by the addition of up to 3 glycosylation sites, wherein the modification is selected from the group consisting of:

Asn³⁰Thr³²;

Asn⁵¹Thr⁵³;

Asn⁵⁷Thr⁵⁹;

Asn⁶⁹;

Asn⁶⁹Thr⁷¹;

Ser⁶⁸Asn⁶⁹Thr⁷¹;

Val⁸⁷Asn⁸⁸Thr⁹⁰;

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Ser⁸⁷Asn⁸⁸Thr⁹⁰;
Ser⁸⁷Asn⁸⁸Gly⁸⁹Thr⁹⁰; (SEQ ID NO: 2);
Ser⁸⁷Asn⁸⁸Thr⁹⁰Thr⁹²;
Ser⁸⁷Asn⁸⁸Thr⁹⁰Ala¹⁶²;
Asn⁶⁹Thr⁷¹Ser⁸⁷Asn⁸⁸Thr⁹⁰;
Asn³⁰Thr³²Val⁸⁷Asn⁸⁸Thr⁹⁰;
Asn⁸⁹Ile⁹⁰Thr⁹¹;
Ser⁸⁷Asn⁸⁹Ile⁹⁰Thr⁹¹;
Asn¹³⁶Thr¹³⁸;
Asn¹³⁸Thr¹⁴⁰;
Thr¹²⁵; and
Pro¹²⁴Thr¹²⁵.

6. (Previously presented) A method of treating disturbances in iron distribution in a patient suffering from non insulin dependent diabetes mellitus comprising administering a therapeutically effective amount of human erythropoietin protein, without administering iron, wherein the protein (EPO) is an analog of SEQ ID NO:1, said analog is selected from the group consisting of: (a) human erythropoietin protein having the amino acid sequence, Ser Ser Ser Ser Lys Ala Pro Pro Pro Ser Leu Pro Ser Pro Ser Arg Leu Pro Gly Pro Ser Asp Thr Pro Ile Leu Pro Gln (SEQ ID NO: 3), extending from the carboxy terminus; (b) the analog in (a) further comprising Ser⁸⁷ Asn⁸⁸ Thr⁹⁰ EPO; (c) the analog in (a) further comprising Asn³⁰ Thr³² Val⁸⁷ Asn⁸⁸ Thr⁹⁰ EPO; (d) the analog in (a) further comprising Gln²⁴ Ser⁸⁷ Asn⁸⁸ Thr⁹⁰ EPO; (e) the analog in (a) further comprising Gln³⁸ Ser⁸⁷ Asn⁸⁸ Thr⁹⁰ EPO; (f) the analog in (a) further comprising Gln⁸³ Ser⁸⁷ Asn⁸⁸ Thr⁹⁰ EPO and (g) darbepoetin alfa.

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7. (Original) The method of claim 1, wherein the erythropoietin protein is pegylated.

8. (Previously presented) A method of treating disturbances in iron distribution in a patient suffering from non-insulin dependent diabetes mellitus comprising administering a conjugate of human erythropoietin protein of SEQ ID NO:1, wherein said conjugate comprising the erythropoietin protein of SEQ ID NO:1 having one to three free amino groups covalently linked to n poly(ethylene glycol) groups of the formula $-\text{CO}-(\text{CH}_2)_x-(\text{OCH}_2\text{CH}_2)_m-\text{OR}$ with the $-\text{CO}$ of each poly(ethylene glycol) group forming an amide bond with one of said amino groups; wherein R is a lower-alkyl; x is 2 or 3; m is from about 450 to about 900; n is from 1 to 3; and n and m are chosen so that the molecular weight of the conjugate minus the erythropoietin protein is from 20 kilodaltons to 100 kilodaltons.

9. (Original) The method of claim 8, wherein x is 3, m is 650 to 750, n is 1 and R is methyl.

10. (Original) The method of claim 8 wherein the conjugate has the formula
$$\text{P}-[\text{NHCO}-(\text{CH}_2)_x-(\text{OCH}_2\text{CH}_2)_m-\text{OR}]_n$$
wherein P is the residue of the protein without the free amino group that forms the amide linkage;

R is lower alkyl;

x is 2 or 3;

m is from about 450 to about 900;

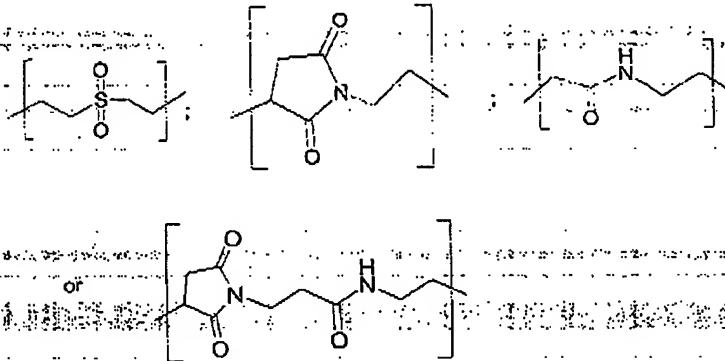
n is from 1-3; and

wherein m and n are selected such that the molecular weight of the conjugate minus the erythropoietin protein is from about 20 kD to about 100 kD.

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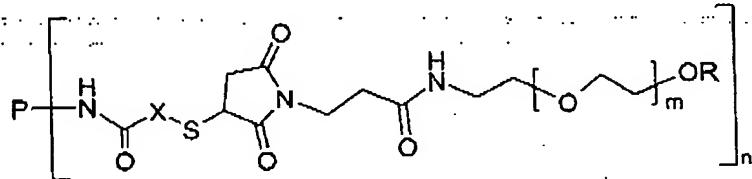
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11. (Currently amended) A method of treating disturbances in iron distribution in a patient suffering from non-insulin dependent diabetes mellitus comprising administering a conjugate of human erythropoietin of SEQ ID NO:1 wherein, said conjugate comprises the erythropoietin protein of SEQ ID NO:1 having one to three free amino groups covalently linked to the erythropoietin protein *via* a linker of the formula -C(O)-X-S-Y- with the C(O) of the linker forming an amide bond with one of said amino groups, X is -(CH₂)_k- or -CH₂(O-CH₂-CH₂)_k-, k is from 1 to 10, Y is



the average molecular weight of each poly(ethylene glycol) moiety is from about 20 kilodaltons to about 40 kilodaltons, and the molecular weight of the conjugate is from about 51 kilodaltons to about 175 kilodaltons.

12. (Previously presented) The method of claim 11, wherein the conjugate has the formula:



wherein n is an integer from 1 to 3; m is an integer from 450 to 900; R is lower-alkyl; X is -(CH₂)_k- or -CH₂(O-CH₂-CH₂)_k-, k is 1 to 10 and P is the residue of

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the erythropoietin protein without the n amino groups which form an amide linkage with X.

13. (Currently amended) A composition for the treatment of disturbances in iron distribution comprising from about 25 to about 2,500 $\mu\text{g}/\text{ml}$ of erythropoietin protein, from about 10 to about 200 mmol/l sulfate, a pharmaceutically acceptable carrier, wherein said composition has and having a pH of from about 6.0 to about 7.0.
14. (Previously presented) The composition of claim 13 comprising from about 50 to about 2,500 $\mu\text{g}/\text{ml}$ of erythropoietin protein, 10 mM sodium phosphate, 40 mM sodium sulfate, 3% mannitol (w/v), 10 mM methionine and 0.01% poloxamer 188 (w/v) and has a pH of about 6.2.
15. (Previously presented) The composition of claim 13 comprising from about 50 to about 2,500 $\mu\text{g}/\text{ml}$ of erythropoietin protein, 40 mM arginine, 30 mM sodium sulfate, 3% mannitol (w/v), 10 mM methionine, 0.01% poloxamer 188 (w/v) and having a pH of about 6.2.
16. (Previously presented) A method of treating disturbances in iron distribution in a patient suffering from diabetes comprising administering a therapeutically effective amount of a composition of human erythropoietin protein of SEQ ID NO:1, wherein the composition comprises from about 25 to about 2,500 $\mu\text{g}/\text{ml}$ of erythropoietin protein, from about 10 to about 200 mmol/l sulfate and having a pH of from about 6.0 to about 7.0.
17. (Canceled)
18. (Canceled)

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19. (Previously presented) A method of treating disturbances in iron distribution in a patient suffering from diabetes comprising administering a therapeutically effective amount of a composition of human erythropoietin protein of SEQ ID NO:1 modified by the addition of up to 3 glycosylation sites, wherein the composition comprises from about 25 to about 2,500 $\mu\text{g/ml}$ of erythropoietin protein, from about 10 to about 200 mmol/l sulfate and having a pH of from about 6.0 to about 7.0.
20. (Canceled)
21. (Previously presented) The method of claim 16, wherein the erythropoietin protein is pegylated.
22. (Previously presented) A method of treating disturbances in iron distribution in a patient suffering from diabetes comprising administering a composition of a conjugate of human erythropoietin protein of SEQ ID NO:1, wherein said conjugate comprises the erythropoietin protein of SEQ ID NO:1 having one to three free amino groups being covalently linked to n poly(ethylene glycol) groups of the formula $-\text{CO}-(\text{CH}_2)_x-(\text{OCH}_2\text{CH}_2)_m-\text{OR}$ with the $-\text{CO}$ of each poly(ethylene glycol) group forming an amide bond with one of said amino groups; wherein R is a lower-alkyl; x is 2 or 3; m is from about 450 to about 900; n is from 1 to 3; and n and m are chosen so that the molecular weight of the conjugate minus the erythropoietin protein is from 20 kilodaltons to 100 kilodaltons, wherein said composition comprises from about 25 to about 2500 $\mu\text{g/ml}$ of erythropoietin protein, from about 10 to about 200 mmol/l sulphate and having a pH of from about 6.0 to about 7.0.
23. (Previously presented) The method of claim 22, wherein x is 3, m is 650 to 750, n is 1 and R is methyl.

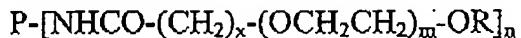
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24. (Previously presented) The method of claim 22, wherein the conjugate has the formula



wherein P is the residue of the protein without the free amino group that forms the amide linkage;

R is lower alkyl;

x is 2 or 3;

m is from about 450 to about 900;

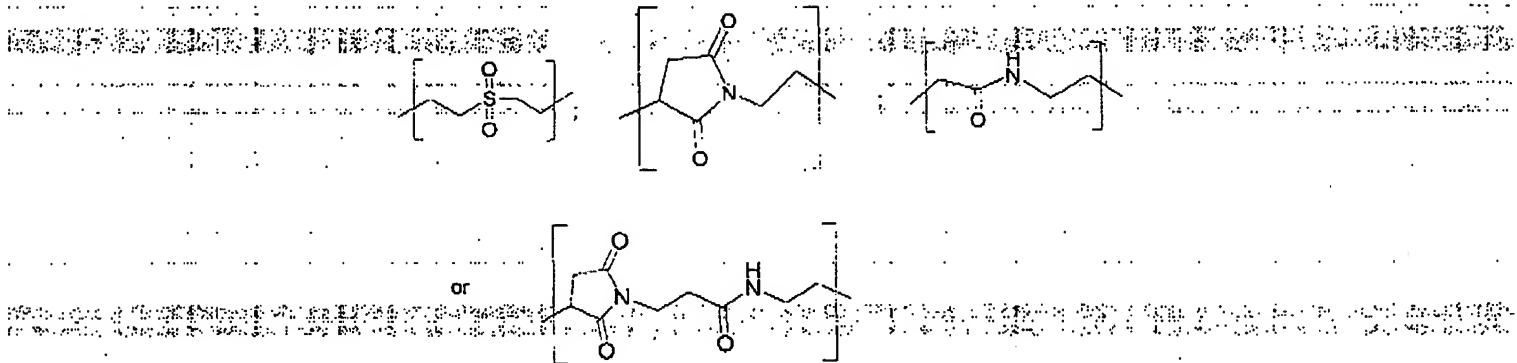
n is from 1-3; and

wherein m and n are selected such that the molecular weight of the conjugate minus the erythropoietin protein is from about 20 kD to about 100 kD.

25. (Currently amended) A method of treating disturbances in iron distribution in a patient suffering from diabetes comprising administering a composition of a conjugate of human erythropoietin protein of SEQ ID NO:1, wherein said conjugate comprising comprises the erythropoietin protein of SEQ ID NO:1 having one to three free amino groups covalently linked to from one to three lower-alkoxy poly(ethylene glycol) groups, each poly(ethylene glycol) group being covalently linked to the erythropoietin protein via a linker of the formula —C(O)-X-S-Y— with the C(O) of the linker forming an amide bond with one of said amino groups, X is -(CH₂)_k or -CH₂(O-CH₂-CH₂)_k-, k is from 1 to 10, Y is

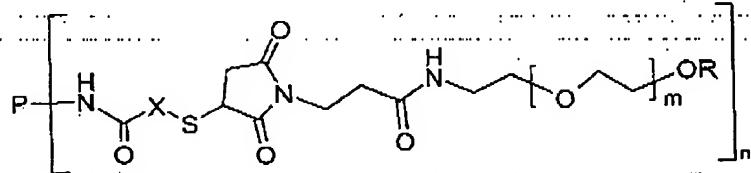
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wherein the average molecular weight of each poly(ethylene glycol) moiety is from about 20 kilodaltons to about 40 kilodaltons, and the molecular weight of the conjugate is from about 51 kilodaltons to about 175 kilodaltons wherein said composition comprises from about 25 to about 2500 ug/ml of erythropoietin protein, from about 10 to about 200 mol/l sulfate and having a pH of from about 6.0 to about 7.0.

26. (Previously presented) The method of claim 25, wherein the conjugate has the formula:



wherein n is an integer from 1 to 3; m is an integer from 450 to 900; R is lower-alkyl; X is -(CH₂)_k- or -CH₂(O-CH₂-CH₂)_k-, k is 1 to 10 and P is the residue of the erythropoietin protein without the n amino groups which form an amide linkage with X.